Muscle cooling delays activation of the muscle metaboreflex in humans

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Ray, Chester A., Keith M. Hume, Kathryn H. Gracey, and Edward T. Mahoney. Muscle cooling delays activation of the muscle metaboreflex in humans. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2436–H2441, 1997.—Elevation of muscle temperature has been shown to increase muscle sympathetic nerve activity (MSNA) during isometric exercise in humans. The purpose of the present study was to evaluate the effect of muscle cooling on MSNA responses during exercise. Eight subjects performed ischemic isometric handgrip at 30% MVC during maximal voluntary contraction to fatigue followed by 2 min of postexercise muscle ischemia (PEMI), with and without local cooling of the forearm. Local cooling of the forearm decreased forearm muscle temperature from 31.8 ± 0.4 to 23.1 ± 0.8°C (P = 0.001). Time to fatigue was not different during the control and cold trials (156 ± 11 and 154 ± 5 s, respectively). Arterial pressures and heart rate were not significantly affected by muscle cooling during exercise, although heart rate tended to be higher during the second minute of exercise (P = 0.053) during muscle cooling. Exercise-induced increases in MSNA were delayed during handgrip with local cooling compared with control. However, MSNA responses at fatigue and PEMI were not different between the two conditions. These results suggest that muscle cooling delayed the activation of the muscle metaboreflex during ischemic isometric exercise but did not prevent its full expression during fatiguing contraction. These results support the concept that muscle temperature can play a role in the regulation of MSNA during exercise.

sympathetic nerve activity; group III and IV afferents; exercise pressor reflex; isometric contraction; muscle ischemia; muscle temperature

The muscle metaboreflexes and mechanoreflexes have been demonstrated to augment muscle sympathetic nerve activity (MSNA) during exercise in humans (13–15, 18). It is believed that stimulation of group III and IV muscle afferents mediate this reflex response. Animal studies have clearly demonstrated the importance of these muscle afferents in eliciting cardiovascular responses (see Ref. 7 for recent comprehensive review of topic).

It has been reported that both group III and IV muscle afferents increase their firing rate when exposed to elevated temperatures (4, 10). Recently, we have shown that MSNA is augmented during ischemic isometric handgrip with elevated muscle temperature (17). These results suggest that heat sensitizes skeletal muscle afferents during muscle contraction in humans and can play a role in the regulation of MSNA during exercise.

The purpose of the present study was to determine the effect of muscle cooling on MSNA during isometric exercise. From the results of our earlier study, we hypothesized that lowering muscle temperature would attenuate exercise-induced increases in MSNA by decreasing the discharge rate of the skeletal muscle afferents. To test this hypothesis, MSNA was recorded during ischemic isometric handgrip before and after local forearm cooling that lowered muscle temperature. The results demonstrate that exercise-induced increases in MSNA are delayed during exercise when muscle temperature is lowered, but the magnitude of the response was similar at fatigue.

METHODS

Eight healthy subjects (5 males and 3 females) were recruited to participate in the study. The subjects were between 20 and 27 yr old. Each subject signed an informed consent after a complete explanation of the testing procedures. The study was approved by the Institutional Review Board.

Experimental protocol. Before the experimental session, each subject performed three maximal handgrips to obtain an average value of maximal voluntary contraction (MVC). All subjects were tested using the dominant arm. Baseline data were collected for 2 min for MSNA, heart rate, arterial blood pressure, and skin and muscle temperature. A pneumatic cuff placed around the upper arm was then inflated to suprasystolic levels (220 mmHg) to induce forearm muscle ischemia. After 1 min of muscle ischemia, the subject performed ischemic isometric handgrip at 30% MVC until fatigue. Fatigue was defined as the point when the subject could not maintain the predefined force (30% MVC) during isometric handgrip. Muscle ischemia was continued for 2 min after the termination of exercise. A 2-min recovery period followed postexercise muscle ischemia (PEMI).

During one trial the exercising forearm was cooled for 30 min before and during the experimental session with the application of two ice packs. Cold was not applied during the control trial. Four subjects performed the cold trial first and four subjects performed the control trial first. The two trials were separated by 30 min of rest.

Measurements. Multifiber recordings of MSNA were made with a tungsten microelectrode inserted in the peroneal nerve. A reference electrode was placed subcutaneously about 2 cm from the recording electrode. Adjustments were made in the placement until a satisfactory site was located. The criteria for acceptable recordings of MSNA were 1) weak electrical stimulation through the electrode elicited involuntary contraction of the appropriate muscles but no paresthesia; 2) tapping of muscles or tendons innervated by the nerve evoked afferent mechanoreceptor discharges, but afferent activity was not elicited by stroking the skin; 3) held expiration increased spontaneous pulse-synchronous bursts of sympathetic impulses; and 4) a sudden arousal stimulus (a loud yell or clap) did not elicit an increase in sympathetic nerve activity.

The nerve signal was amplified, fed through a band-pass filter with a bandwidth of 700–2,000 Hz, and passed through a resistance-capacitance integrating network with a time

H2436 0363-6135/97 $5.00 Copyright © 1997 the American Physiological Society
constant of 0.1 s to obtain a mean voltage display of the nerve activity. The mean voltage was routed through a loudspeaker as well as to an on-line computer for monitoring and data analysis purposes throughout the experimental session. Sympathetic bursts were identified by inspection of the mean voltage neurogram. MSNA was expressed as burst frequency (bursts 30 s−1) and total activity. Total activity was calculated as the sum of the areas of the bursts for a given 30-s interval.

Skin thermisters were used to measure skin surface temperature. Two thermisters were placed between the skin and the ice pack (one proximal and one distal) on the cooled forearm. A third thermister was placed on the contralateral forearm to serve as a control. Each skin thermister was insulated with a small piece of foam. Muscle temperature was recorded in seven subjects every 30 s from a muscle temperature probe (YSI 552, Yellow Springs, OH) inserted into the flexor muscles of the exercising forearm.

A Finapres blood pressure monitoring unit was used to measure heart rate and arterial blood pressure. The photoplethysmographic cuff was placed on the middle finger of the nonexercising arm. Borg's numerical scale from 6 to 20 was used to monitor the participant's perception of exertion at fatigue (1; n = 4).

Data analysis. MSNA, heart rate, arterial pressure, and skin and muscle temperature were determined for each 30 s of the first 2 min of the experimental protocol and then at fatigue and PEMI. Data were analyzed using a two-within factor repeated analysis of variance (time and condition (cold, control)). Tests for simple effects were done when the interaction term was found to be significant. Statistical significance was accepted at P < 0.05. All values are means ± SE.

RESULTS

Time to fatigue was unaffected by local cooling. Fatigue time was 156 ± 11 s and 154 ± 5 s for control and cold, respectively. Ratings of perceived exertion at fatigue were 18 ± 2 for both trials. There were no significant differences between preexercise values obtained before and after forearm muscle ischemia.

Skin and muscle temperature responses to forearm cooling are shown in Fig. 1. Skin temperature was reduced by local cooling (28.4 ± 0.6 to 17.9 ± 1.0°C). Resting muscle temperature was decreased by cooling from 31.8 ± 0.4 to 23.1 ± 0.8°C. Skin and muscle temperature during exercise decreased slightly during the cold trial (P < 0.05) but not during the control trial.

Heart rate and arterial pressure responses to the experimental protocols are shown in Fig. 2. Heart rate increased during exercise, but the responses were not generally affected by local cooling (P = 0.8 for time × condition interaction). However, heart rate tended to be higher during the second minute of exercise (P = 0.053). During PEMI, heart rate returned to baseline for both conditions (control and cold, Fig. 2). Arterial pressure increased with exercise, but cooling had no effect on systolic, diastolic, or mean arterial pressure responses to exercise. Arterial pressure remained significantly elevated during PEMI, but there was no difference between conditions (Fig. 2).

MSNA responses are shown in Fig. 3. Cooling had no effect on resting MSNA. MSNA, expressed as burst frequency and total activity, increased during exercise. MSNA responses were attenuated with muscle cooling during the second minute of exercise. Total activity was lower in the cold trial at both 90 and 120 s of exercise (P = 0.01 and P = 0.008, respectively). Burst frequency was lower during the cold trial at 90 s of exercise (P = 0.02). However, there was no difference between trials at fatigue or during PEMI for either expression of MSNA (Fig. 3).

DISCUSSION

The purpose of the present study was to evaluate the effect of muscle cooling on MSNA responses to isometric exercise. The main findings of this study were 1) local forearm cooling, which decreased muscle temperature, had no effect on resting MSNA; 2) decreased muscle temperature delayed exercise-induced increases in MSNA; and 3) decreased muscle temperature did not effect MSNA responses at fatigue and during PEMI. We will discuss these findings and examine the possible mechanisms by which exercise-induced increases in MSNA were delayed by muscle cooling.

Few animal studies have examined skeletal muscle afferent responses to muscle cooling. Hertel et al. (4) showed both group III and IV muscle afferents to be...
cold sensitive. Cold thermosensitivity was indicated by a decrease in discharge frequency of these afferents in response to a decrease in muscle temperature from 36 to 29°C. Similarly, Kumazawa and Mizumura (10) reported sensitivity to cooling with a suppressed discharge in ~50% of fibers tested at a muscle temperature below 22°C. In contrast, these investigators found that both group III and IV muscle afferents increase their rate of firing to increases in temperature (4, 10). However, all of these animal studies examining the effect of muscle temperature on muscle afferents were done with resting muscle. We believe this study represents the first description of the interaction between decreased muscle temperature and contraction and its resultant effect on cardiovascular and sympathetic responses.

Muscle cooling and resting MSNA. Little data exist regarding the relationship between muscle temperature and resting MSNA in humans. Kregel et al. (9) found a dissociation between MSNA and hand muscle temperature during and after a cold pressor test. The present study demonstrates that an 8°C decrease in forearm muscle temperature fails to alter resting MSNA. Previously, we have shown that elevating muscle temperature of the forearm does not change resting MSNA (17). It should be recognized that our findings and those of Kregel et al. (9) are the result of changing temperature of only a small muscle mass (i.e., forearm or hand). Therefore, the possibility remains that altering muscle temperature in a larger muscle mass may evoke changes in resting MSNA.

Muscle cooling and MSNA responses to exercise. Exercise-induced increases in MSNA were delayed with local cooling. This result indicates that cooling the exercising muscle can alter MSNA responses. There was no difference in MSNA during the first minute of isometric exercise, suggesting that muscle cooling had no effect on the muscle mechanoreflexes. If the muscle mechanoreflex was responsible for the delay of exercise-induced increases in MSNA, it would be expected that this effect would be present during the entire exercise bout. However, this was not the case because MSNA was similar during the first minute of exercise and at fatigue in both trials. The similar MSNA response at fatigue was unlike what we observed during muscle heating (17). Elevation of muscle temperature augmented MSNA during the first minute of isometric handgrip and throughout the remainder of fatiguing exercise.

What was the mechanism for the delay of MSNA during exercise with muscle cooling? One explanation for this finding is that muscle cooling delayed the onset of the muscle metaboreflex. It has been shown that there is a time delay in the increase of MSNA with isometric exercise (13, 18). This time delay is a function of the exercise intensity and development of muscle fatigue (23, 24). It is generally believed that until there is a sufficient buildup of metabolites in the interstitia
space the metaboreflex will not be activated. Edwards et al. (2) showed an attenuation of muscle metabolism during isometric exercise with local muscle cooling. Decreased muscle temperature resulted in a significant reduction in lactate production during fatiguing isometric exercise. It has been shown that lactic acid augments group IV afferent discharge (21, 26). Also, Ettinger et al. (3) reported attenuated MSNA responses to isometric handgrip and PEMI when lactic acid production was decreased by dichloroacetate. Muscle acidosis has been associated with increases in MSNA and calf vascular resistance during exercise (16, 25, 27). Thus the decrease in muscle metabolism, specifically glycolysis during nonfatiguing exercise, is a possible explanation for the delay in metabolite accumulation observed in the present study. However, MSNA at fatigue and during PEMI was not different between the control and cold trials, suggesting that muscle cooling did not prevent the full expression of the muscle metaboreflex.

The delay in the increase of MSNA during the second minute of exercise may be related to a decrease in the discharge frequency of chemically sensitive muscle afferents. As previously stated, it has been shown that lowering muscle temperature decreases the discharge rate of muscle afferents (4, 10). However, this effect is only transient because exercise-induced increases in MSNA were comparable at fatigue. It would seem likely that when marked changes in the chemical milieu of the interstitial space occur, as during fatiguing contractions, chemical stimulation of the muscle afferents would overwhelm any influence that cooling may have on muscle afferent discharge.

Other possible mechanisms for the delay in exercise-induced increases in MSNA during handgrip include central command and baroreflexes. Neither appears to contribute to the delay in the MSNA response during exercise. Central command has been shown to increase MSNA only during intense bouts of exercise (19, 28, 30). In our study, cooling caused less rather than more MSNA during the second minute of exercise, before fatigue or intense volitional effort would have occurred. Baroreflexes would not be expected to cause the attenuation in MSNA because arterial pressure was not different during the cold and control trials. Thus arterial pressure would not be expected to engage the arterial baroreflex to a greater extent during the cold trial (22). Likewise, the cardiopulmonary baroreflexes would not be expected to be changed by cooling only the forearm. If cooling did have an effect on the cardiopulmonary baroreflexes, MSNA would have been less at rest.

Because skin temperature remained relatively constant during exercise with cooling, it is unlikely that simulation of cutaneous cold receptors was responsible for the attenuation of MSNA during exercise. Additionally, it is unlikely that cutaneous nociceptive afferents mediated the delay in exercise-induced MSNA. The lack of change in resting MSNA when skin temperature was decreased during baseline and the first minute of exercise argues against a role of cutaneous receptors.

![Fig. 3. Muscle sympathetic nerve activity (MSNA), expressed as burst frequency (top) and total activity (bottom), during first 2 min of exercise (30-s intervals), fatigue, and PEMI. ∗P < 0.05 vs. cold.](http://ajpheart.physiology.org/Downloadedfrom/10.1152/ajpheart.00249.2017)
The failure to see lower arterial pressure during the second minute of exercise with muscle cooling when MSNA responses were lower was surprising. However, it is possible that sympathetic outflow to other vascular beds may have increased to compensate for the decrease in MSNA. Kregel et al. (8) reported differential sympathetic outflow to thermal stress. Additionally, it has been demonstrated that cooling of blood vessels increases the vasoconstrictor response to sympathetic nerve stimulation (12, 20). This greater vasoconstrictor response has been attributed to increased affinity of α-adrenergic receptors for norepinephrine (5) and decreased neuronal uptake of norepinephrine (11), which would result in an increased concentration of norepinephrine in the vicinity of the adrenergic receptors on the smooth muscle cell. Thus this greater vasoconstrictor response may have offset the decrease in sympathetic outflow to skeletal muscle observed in the current study and provide another explanation for the lack of change in blood pressure. However, this would not explain the similar arterial pressure response at fatigue. Finally, the decrease in MSNA elicited by muscle cooling may have not been of sufficient magnitude to change arterial pressure. It might be speculated that if a larger exercising muscle mass was involved, differences in MSNA may have been greater and changes in arterial pressure would have been observed.

We have demonstrated, in this study and an earlier study (17), that alterations in muscle temperature can change exercise-induced MSNA responses. These studies demonstrate a possible important mechanism by which cardiovascular function is regulated during exercise. In addition to mechanical and metabolic changes within the exercising muscle that have been considered important in providing feedback to the cardiovascular control centers, alterations in muscle temperature appear to provide an additional feedback signal. Whether this effect is direct by modifying muscle afferent sensitivity or is indirect by altering muscle metabolism remains unclear.

We chose to use ischemic forearm exercise to eliminate possible changes in blood flow to the forearm induced by the cold. Johnson et al. (6) found no effect of arm heating on forearm blood flow at rest. However, the effect that local cooling of a small muscle mass, as in this study, would have on muscle blood flow during exercise is unknown. Blocking blood flow to the forearm prevented the delivery of warmer blood to and the removal of cooler blood from the forearm muscle. Ischemic exercise eliminated any possible contribution that other thermal receptors throughout the body could have made to our results. Additionally, by blocking blood flow to the forearm, the contribution of oxidative metabolism was minimized. Although the experimental exercise model is unlike normal dynamic exercise, the findings from the current and previous study (17) should have relevance to dynamic exercise. In both modes of exercise, muscle afferent activity plays a major role in regulating MSNA (13, 29). Moreover, marked changes in muscle temperature occur with dynamic exercise.

In conclusion, the present study indicates that decreases in muscle temperature can contribute to an attenuation of MSNA before fatigue during isometric exercise. The data suggest that this effect is mediated by a delay in the activation of the muscle metaboreflex. These results support the concept that muscle temperature can play a role in the regulation of MSNA during exercise.

This project was supported by a grant-in-aid from the American Heart Association, Georgia Affiliate, and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-44571.

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Received 4 April 1997; accepted in final form 24 July 1997.

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